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(54) VITELLOGENIN ORIGINATING FROM CYPRINODONTIFORMES AND ANTIBODY

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method useful for evaluating estrogen like activity of a chemical substance capable of determining the quantity of vitellogenin in a male animal body exposed to the chemical substance.
SOLUTION: The vitellogenin originating from Cyprinodontiformes having amino acid sequence expressed by sequence number 1, and an antibody, etc., against the vitellogenin.

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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] BITEROJIENIN of the Cyprinodontiformes animal origin which has the amino acid sequence shown by the array number 1.
[Claim 2] The antibody to BITEROJIENIN according to claim 1.
[Claim 3] The quantum approach of BITEROJIENIN in the sample which carries out the quantum of the antigen antibody complex which the sample and the antibody according to claim 2 were made to react, and was produced.
[Claim 4] The approach according to claim 3 of carrying out the quantum of the antigen antibody complex with enzyme immunoassay.
[Claim 5] The approach according to claim 3 or 4 a sample is a sample of the body grinding object origin of a male cyprinodont.
[Claim 6] The kit for BITEROJIENIN detection containing an antibody according to claim 2.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the antibody to BITEROJIENIN and this BITEROJIENIN of the cyprinodont origin.
[0002]
[Description of the Prior Art] It is reported that some chemicals in an environment show estrogen Mr. activity in recent years. An operation of this chemical loses human hormone balance, and since it is apprehensive about becoming the cause of a disease, the attempt which measures the estrogen Mr. activity of a chemical as part of the safety assessment of a chemical is made. BITEROJIENIN is the precursor of the quality of phosphoprotein contained in the yolk, and is detected for animals, such as the animal of oviparity, i.e., fishes, an amphibian, birds, and Insecta. In the liver of a female animal, BITEROJIENIN gene expression is promoted, and BITEROJIENIN is compounded in large quantities by estrogen and secreted in blood. On the other hand, for a normal male animal, although most BITEROJIENIN is not compounded, the composition is guided by estrogen administration. Then, in order to evaluate the estrogen Mr. activity of a chemical, it is anxious for development of the approach of carrying out the quantum of BITEROJIENIN of the inside of the body of a male animal exposed to the chemical as a useful approach.
[0003]
[Means for Solving the Problem] Under this situation, as a result of inquiring wholeheartedly, this invention persons succeeded in isolating BITEROJIENIN from the cyprinodont which is the model animal of an aquatic animal, and producing the antibody to this, and resulted in this invention. That is, this invention is BITEROJIENIN (it is hereafter described as this invention protein.) of the Cyprinodontiformes animal origin which has the amino acid sequence shown by 1 array number 1.
2) Make the antibody (it is hereafter described as this invention antibody.), and three samples and this antibody to this BITEROJIENIN react, and offer the BITEROJIENIN checking kit containing the quantum approach of BITEROJIENIN in the sample which carries out the quantum of the produced antigen antibody complex, and 4 this invention antibody.
[0004]
[Embodiment of the Invention] Hereafter, this invention is explained to a detail. this invention protein is BITEROJIENIN of the fishes animal origin belonging to Cyprinodontiformes, and has the amino acid sequence shown by the array number 1. this invention protein can be obtained from the fishes animal belonging to Cyprinodontiformes, such as HIMEDAKA, by the following approach. For example, HIMEDAKA etc. is medicated with estrogen, such as beta-estradiol and a diethyl still BESUTE roll, for about one to two weeks, and composition of BITEROJIENIN is guided. As a medication method, a food intake may be mixed and carried out to food, and said estrogen may be added in the water used for breeding. Ascites is collected from the cyprinodont in which ascites accumulated by exposure of such estrogen using a capillary tube etc. Subsequently, a gel filtration column chromatography refines BITEROJIENIN from this ascites, for example. The approach of performing by putting pressures, such as FPLC besides an open column or HPLC, can also be used for this chromatography, and you may use for it combining these. Such various actuation of a column chromatography is new chemistry experiment lecture 1. protein I. Edited by Japanese Biochemical Society It can carry out according to the approach indicated by Tokyo Kagaku Dojin (1990) etc. Furthermore, it can also refine combining the other purification approaches used in purification of the usual protein, for example, an ion-exchange column

chromatography, a hydrophobic column chromatography, etc. if needed.
[0005] The fraction in which this invention protein is contained can select the molecular weight of for example, this protein against an index. it be new chemistry experiment lecture 1. protein I about the elution fraction in the above purification processes in order to measure proteinic molecular weight. edited by Japanese Biochemical Society after process with the sample processing liquid containing reducing agents, such as beta-mercaptoethanol and dithiothreitol, according to the usual approach indicate by Tokyo Kagaku Dojin (1990) etc., it dissociate by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (it be hereafter describe as SDS-PAGE.), and gel be dye by the usual protein staining technique. By comparing the location of the band detected with the location of the band of molecular weight marker protein, the molecular weight of the appearance under the reduction conditions of this invention protein can be measured. As molecular weight marker protein, for example A myosin (molecular weight 220kDa), A phosphorylase (97.2kDa), bovine serum albumin (66kDa), Ovoalbumin (46kDa), carbonic anhydrase (30kDa), Trypsin The mixture (Amersham Pharmacia biotechnology theque incorporated company make) of inhibitor (21.5kDa) and a lysozyme (14.3kDa), A myosin (molecular weight 200kDa), beta-galactosidase (116kDa), The mixture (the first chemicals incorporated company make) of bovine serum albumin (66kDa), aldolase (42.4kDa), carbonic anhydrase (30kDa), and a myoglobin (17.2kDa) etc. can be used. The molecular weight of the appearance under the reduction conditions of this invention protein Electrophoresis is carried out by polyacrylamide gel SDS-10 to 20%. As molecular weight marker protein A myosin (molecular weight 220kDa), A phosphorylase (97.2kDa), bovine serum albumin (66kDa), Ovoalbumin (46kDa), carbonic anhydrase (30kDa), Trypsin If inhibitor (21.5kDa) and a lysozyme (14.3kDa) are used, it will be measured with about 170 kDa(s). Electrophoresis is carried out by SDS-7.5% polyacrylamide gel. As molecular weight marker protein A myosin (molecular weight 200kDa), If beta-galactosidase (116kDa), bovine serum albumin (66kDa), aldolase (42.4kDa), carbonic anhydrase (30kDa), and a myoglobin (17.2kDa) are used, it will be measured with about 200 kDa (s).
[0006] The partial amino acid sequence of this invention protein is new chemistry experiment lecture 2. protein II. Edited by Japanese Biochemical Society According to the usual approach indicated by Tokyo Kagaku Dojin (1990) etc., it can determine using a peptide sequencer etc. this invention protein by which isolation purification is carried out from ascites has the amino acid sequence shown in the amino terminus by the array number 1.
[0007] this invention antibody can be prepared by using as an antigen the partial fragment of this invention protein prepared as mentioned above or this invention protein, and carrying out immunity of the animal. In order to fry the animal of birds, such as the mammals, such as a mouse, a hamster, a guinea pig, a rat, a rabbit, a dog, and a goat, and a fowl, and to carry out immunization of these animals as an animal which carries out immunization, for example, it is J.ASSOC.OF.ANAL.CHEM 70 (6). 1025-1027 etc. is medicated with an antigen once or more using the usual approach indicated (1987). Specifically, a medicine will be preferably prescribed for the patient 2 to 3 times at intervals of ten - 21 days on seven to the 30th. A dose makes a standard about 5mg of abbreviation per [0.05 / about] time (for example, an antigen) -. A route of administration can choose hypodermic administration, intracutaneous administration, intraperitoneal administration, intravenous administration, intramuscular administration, etc., and is an administration gestalt with a desirable injection performed intraperitoneal or hypodermically in a vein. In this case, in addition, the buffer solution with a suitable antigen, for example, CFA, (Complete Freund'sAdjuvant), RAS[MPL(Monophosphoryl Lipid A)+TDM(Synthetic TrehaloseDicorynomycolate)+CWS (Cell Wall Skeleton) adjuvant system] Or although dissolved and used for a sodium system phosphate buffer solution or a physiological saline containing one sort of adjuvants usually used, such as an aluminum hydroxide, etc., the above adjuvants may be used neither according to a route of administration nor conditions. An adjuvant means the matter which reinforces the immunoreaction to the antigen nonspecific here, when a medicine is prescribed for the patient with an antigen. And after breeding the animal which prescribed the above-mentioned antigen for the patient about two weeks to about about 12 weeks, the little sampling of the blood of this animal is carried out from a vein etc., and the antibody titer is measured. If antibody titer rises, according to a situation, count operation of the administration of an antigen of suitable will be carried out further. this invention antibody can be obtained as poly KURONARU antiserum by extracting blood by the usual approach of the last administration from this animal behind, and separating and refining this blood about one week to

about about eight weeks, by the usual approaches, such as chromatographies, such as precipitation by using centrifugal separation, an ammonium sulfate, or a polyethylene glycol, gel filtration chromatography, an ion exchange chromatography, and an affinity chromatography. Into this blood serum, a complement system may be inactivated by processing for 30 minutes at 56 degrees C. By isolating an immune competence B cell from the above-mentioned animal which carried out immunization, uniting this immune competence B cell and the tumor cell which can carry out cell division continuously, and isolating and selecting the fusion object to generate, the hybridoma cell which produces a desired peptide antibody is cloned, and this invention antibody can also be obtained as a mono-KURONARU antibody by cultivating this hybridoma cell in the living body within a test tube.
[0008] this invention antibody prepared as mentioned above can be made to be able to act on sample offering protein, and the protein in which this antibody and an antigen-antibody reaction are shown can be detected. For example, the amount of BITEROJIENIN in this sample can be measured by mixing the sample prepared from this invention antibody and the body of fishes animals, such as a cyprinodont, making an antigen-antibody reaction perform, and carrying out the quantum of the produced antigen antibody complex. The quantum of an antigen antibody complex is the new chemistry experiment lecture 12. molecule immunology III. Edited by Japanese Biochemical Society Tokyo Kagaku Dojin (1992), E., Harlow, D., Lane work, an antibody; it can carry out according to the immunoassay indicated in a laboratory manual (Antibodies;A Laboratory Manual), the Cold Spring Harbor Laboratory issue (Cold Spring Harbor Laboratory press), 1988, etc. Specifically, the following approaches can be raised.
(1) To the solid-state supporting material with which it comes to combine this invention antibody (the first antibody) directly or indirectly After adding the sample of the body origin of fishes animals, such as a cyprinodont which is a specimen, and performing an antigen-antibody reaction, an unreacted object -- washing and removal -- carrying out -- (2) -- the unreacted object after adding this invention antibody (second antibody) by which the indicator was carried out to the solid-state supporting material concerned and performing an antigen-antibody reaction -- washing and removal -- carrying out -- (3) -- the amount of the labelled antibody (second antibody) subsequently to this solid-state supporting material fixed is measured. this invention antibody (the first antibody) prepared as mentioned above is added on 96 hole micro titer plates, such as Nunc-Immuno Plate MaxiSorp (Nunc trademark), and it is more specifically left from about 1 hour at about 4 to about 37 degrees C on them for about about 20 hours so that it may become about 500 ng/well extent from about 50 ng(s) as an amount of protein. then, about 100microl of the phosphate buffer solution (pH=7.4) of about 10 mM extent which contains the sodium chloride of about 140 mM(s), and about 0.1%Tween 20 for this plate from -- about 500microl It washes about 5 times from 2 times. furthermore, phosphate buffer solution (pH=7.4) about 100microl of about 10 mM extent which contains the sodium chloride of about 140 mM(s), and about 1% cow serum albumin for blocking from -- about 500microl -- adding -- about 4 to about 37 degrees C -- the about 60-minute about room from for about 30 minutes -- it is left. then, phosphate buffer solution (pH=7.4) about 100microl of about 10 mM extent which contains the sodium chloride of about 140 mM(s), and about 0.1%Tween 20 for this plate from -- about 500microl It washes about 5 times from 2 times. thus, the samples (for example, a bodily grinding object, blood, body fluid, etc.) of the body origin of fishes animals, such as a cyprinodont which responded to the processed plate at the need, and was diluted and prepared with distilled water, the buffer solution, a physiological saline, etc., -- about 50microper hole l from -- about 200microl adding -- room temperature - an antigen-antibody reaction is performed by leaving it from about 1 hour at about 37 degrees C for about 2 hours. then, phosphate buffer solution (pH=7.4) about 100microl of about 10 mM extent containing the sodium chloride of about 140 mM(s), and about 0.1%Tween 20 from -- it washes about 5 times from 2 times by about 500microl. the next -- this plate -- for example, the phosphate buffer solution (pH=7.4) (about 5 mM(s) - about 50 mM extent) of about 10 mM extent which contains the sodium chloride of about 140 mM(s), and about 0.1%Tween 20 for the biotin-ized this invention antibody (second antibody) -- diluting -- about 50microl from -- about 200microl addition -- carrying out -- room temperature - an antigen-antibody reaction is performed by leaving it from about 1 hour at about 37 degrees C for about 2 hours. then, phosphate buffer solution (pH=7.4) about 100microl of about 10 mM extent containing the sodium chloride of about 140 mM(s), and about 0.1%Tween 20 from -- about 500microl An unreacted object is removed by washing about 5 times from 2 times. It ranks second. for example, the horseradish origin peroxidase (commercial item of Streptavidin-horseradish peroxidase conjugate:, for example, Amersham) by which the streptoavidin

indicator was carried out -- about 50microl About 200microl addition is carried out. from -- After leaving it for about 60 minutes after for about 30 minutes at a room temperature - 37 degrees C of abbreviation and performing the same washing actuation as the above, a reaction substrate solution (for example, a 4mM o-phenylenediamine and 0.004% hydrogen peroxide --) 0.02M According to the amount of an antigen (namely, BITEROJIENIN contained in a sample), brown coloring is obtained by adding a citric acid and 0.05M Na2HPO4/pH5.0, and leaving it for about 60 minutes after for about 10 minutes at a room temperature - 37 degrees C of abbreviation. The amount of BITEROJIENIN in a sample can be measured by measuring the absorbance in the substrate specific wavelength (in for example, the case of o-phenylenediamine 490-492nm) of this using spectrophotometers, such as a microplate reader for 96 hole micro titer plates. In addition, it is good for the quantum of BITEROJIENIN in a sample to measure similarly using the purification preparation of BITEROJIENIN and to produce a calibration curve. When other protein tends to measure the amount of BITEROJIENIN in this sample like the grinding object of the body of fishes animals, such as a cyprinodont, using the sample which exists so much in addition to BITEROJIENIN made into the purpose, a calibration curve is also good to produce under existence of such protein.

[0009] As a kit for BITEROJIENIN detection containing this invention antibody which can be used for measurement of the above amounts of BITEROJIENIN, the following configurations can be raised, for example.

(1) Solid-state supporting material with which it comes to combine this invention antibody (the first antibody) directly or indirectly.

(2) The reagent containing this invention antibody (second antibody) by which the indicator was carried out.

It is (3) still more auxiliary. Purification BITEROJIENIN and (4) The buffer solution and (5) Additives, such as a polypeptide, a surfactant, etc. which prevent formation of nonspecific adsorption and floc, and (6) pipets, a reaction container, a count curve, etc. may be included. The above-mentioned solid-state supporting material can have a configuration which is very different according to the specific purpose meant on the occasion of use. For example, a pan, a ball, a plate, a small rod, a cel, a small bottle, a small tube, a fiber, a network, etc. can be raised. A corpuscle, a tube, or a rod etc. which consists of the micro titer plate, polystyrene, and polystyrene latex which consist of transparence plastic material, for example, a polyvinyl chloride, or polystyrene as a concrete example can be raised, and 96 hole micro titer plate made from polystyrene can more specifically be raised. And in order to combine this invention antibody (the first antibody) with such a solid-state supporting material directly or indirectly, solid-state supporting material is beforehand activated by the usual approach using glutaraldehyde or a cyanogen bromide. Subsequently, it adds and the antibody joint liquid which contains this invention antibody in the solid-state supporting material activated by doing in this way is set fixed time. The phosphate buffer solution (pH=7.4) of about 10 mM(s) which contain this invention antibody by about 5microg [about 0.05microg/ml to //ml] concentration, and contain the sodium chloride of 140mM as antibody joint liquid used here, for example etc. can be raised. Moreover, as the processing time, 24 hours can be raised, for example from about 1 hour. When combining this invention antibody (the first antibody) with solid-state supporting material indirectly, it is good to combine solid-state supporting material and this invention antibody (the first antibody) through the amount carrier molecule of macromolecules which is not recognized by the spacer and/or the antibody. Here, the amount carrier molecule of macromolecules which is not recognized by the first antibody is the amount carrier molecule of macromolecules which is not used in manufacture of this antibody. This is important when the first antibody is a polyclonal. this invention antibody (second antibody) by which the indicator was carried out For example, an antibody; laboratory manual (Antibodies;A Laboratory Manual), The Cold Spring Harbor Laboratory issue (Cold Spring Harbor Laboratory press), It applies to the technique indicated in 1988 etc. correspondingly. For example, a peroxidase, The alkaline phosphatase, beta-D-galactosidase, glucose oxidase, It can obtain by carrying out the indicator of this invention antibody with enzymes, such as glucoamylase, carbonic acid anhydrase, acetylcholineaterase, a lysozyme, a malate dehydrogenase, and a glucose-6-phosphate dehydrogenase, etc. In using such the second antibody for measurement, after adding this antibody to the solid-state supporting material with which it comes to combine the first antibody and an antigen, incubating and washing removes the second antibody in the condition of isolation, the second antibody fixed by solid-state supporting material is detectable by making the substrate of the above-mentioned

marker enzyme act, and measuring a reaction by coloring etc. For example, if a hydrogen peroxide is used as a substrate and diaminobenzidine or o-phenylenediamine is used as a color reagent when the indicator of the second antibody is carried out by the peroxidase, brown or yellow will be produced. Moreover, when the indicator of the second antibody is carried out by glucose oxidase, it is 2 and 2'-acid-G (a 3-ethylbenzo thiazoline-6-sulfonic acid (ABTS) etc. can be used.) as a substrate. Moreover, this invention antibody (second antibody) by which the indicator was carried out may be obtained by carrying out the biotin label for example, of this invention antibody (the first antibody) using a "protein biotin-ized system" (product made from Amersham). When using such the second antibody for measurement After adding this antibody to the solid-state supporting material with which it comes to combine the first antibody and an antigen and incubating, The enzyme which carried out the indicator by the matter (streptoavidin) specifically combined with the above-mentioned biotin after washing removed the second antibody in the condition of isolation, For example, a peroxidase, alkaline phosphatase, beta-D-galactosidase, Glucose oxidase, glucoamylase, carbonic acid anhydrase, Acetylcholineaterase, a lysozyme, a malate dehydrogenase, A glucose-6-phosphate dehydrogenase etc. can be combined with the second antibody, a substrate can be made to be able to act like the above, and the second antibody fixed by solid-state supporting material can be detected by measuring a reaction by coloring etc.

[0010] The measuring method of the above amounts of BITEROJIENIN is applicable to evaluation of the estrogen Mr. activity of an examined substance. For example, after making the male of cyprinodonts, such as HIMEDAKA, expose to an examined substance, a test sample, for example, a bodily grinding object etc., is prepared from the body, and the amount of BITEROJIENIN in this sample is measured by the above approaches using this. When BITEROJIENIN of high concentration is intentionally detected in the individual exposed to the examined substance as compared with the BITEROJIENIN concentration at the time of examining similarly about the individual which is not made to expose to the chemical which has estrogen Mr. activity, it is suggested that this examined substance has estrogen Mr. activity.

[0011]

[Example] Hereafter, this invention is not limited by these examples although an example explains this invention to a detail further.

Example 1 (purification of BITEROJIENIN)

(1) The purification beta-estradiol (the Wako Pure Chem Industries make, purity 97.0-103%) of BITEROJIENIN was added as a bait (carp for fries) so that it might become 0.1, 1.0, and 10 mg/g food, and after adding the acetone to this and mixing, the acetone was removed under ventilation of a hair dryer. in this way, the amount of gluttony of the prepared food -- a female -- an adult and a male -- an adult -- it gave HIMEDAKA 30 individuals each for 13 days by 1 time per of frequency day. Moreover, beta-estradiol (the Wako Pure Chem Industries make, purity 97.0-103%) was dissolved in dimethylformamide, it added in dechlorination water and beta-estradiol water solution was prepared so that it might become 0.01, 0.1, and 1.0 mg/l. the inside of this water solution -- a female -- an adult -- HIMEDAKA 15 individual was bred for ten days, and this water solution was exchanged by three frequency/week in the meantime. HIMEDAKA 32 individual (male 25 individual, Metz 7 individual) which survived even after being exposed to beta-estradiol in the two above-mentioned approach was made an incision in the abdomen, ascites was extracted by the capillary tube, and the ascites of a total of 400microl was obtained. When the protein concentration of the obtained ascites was measured using the dye reagent stain solution (product made from Bio-Rad), they were 9.2 mg protein / ml. They are this ascites 1, 1/5, 1/10, 1/20, and 1/40microl SDS-10-20% It analyzed by polyacrylamide gel electrophoresis (the gel by first chemicals incorporated company is used). As molecular weight marker protein, they are a myosin (molecular-weight 220kDa), a phosphorylase (97.2kDa), bovine serum albumin (66kDa), ovoalbumin (46kDa), carbonic anhydrase (30kDa), and a trypsin. The molecular weight marker (Amersham Pharmacia biotechnology theque incorporated company make) which consists of inhibitor (21.5kDa) and a lysozyme (14.3kDa) was migrated. The protein band of six-kind remainder was detected on the lane which migrated the above-mentioned ascites. When the location of the detected band was compared with the migration location of the band of molecular weight marker protein, the proteinic band existed in the location equivalent to about 170 kDa(s), and it was presumed that this was equivalent to BITEROJIENIN of HIMEDAKA. It dilutes so that the following gel-filtration buffer may be added to the aforementioned ascites and protein concentration may become [ml] in 0.5mg /, and it is this diluted sample 1 ml. It hung on the Sepharose 6B gel-filtration column (the product made from Sigma, column

size: 1.6X50 cm), and was eluted in the gel-filtration buffer on condition that the following.
a) Gel-filtration buffer : 0.02 M Tris-HCl, pH 8.0, 2% NaCl, and 0.1% NaN3b Rate of flow: 15ml (a part for 0.25 ml/)/hour
c) Temperature: It isolated preparatively 1.9ml of 4-degree-C eluates at a time, the protein quantum of each fraction was carried out, and the fractions (fraction 27-35) equivalent to the first protein peak were collected. Next, after dialyzing this fraction, removing a salt and condensing with freeze drying, It is 0.1M so that protein concentration may become the obtained concentration liquid (7.8mg protein / ml) with 5 mg/ml. A phosphoric-acid buffer is added. The 20microl is hung on a TSK-GEL G3000SW column (the TOSOH CORP. make, column size:7.8 X 300 mm), and 0.3M NaCl is included on condition that the following. It was eluted in the 0.1M phosphoric-acid buffer. An elution pattern is shown in drawing 1 .
a) Rate of flow: 0.5 ml/minb Column **: 30 kg/cm2c Detector: UV 280 nmd Temperature: The eluate for 9.5 minutes - 20-degree-C holding-time 16.5 minutes was isolated preparatively. When a part of eluate isolated preparatively was analyzed SDS-10 to 20% by polyacrylamide gel electrophoresis (the gel by first chemicals incorporated company is used), it turned out that this sample is refined by the high grade. Then, after dialyzing and condensing this sample, distilled water was added so that protein concentration might be set to about 3 mg/ml at this, and it used for future experiments as purification BITEROJIENIN.
[0012] (2) The molecular weight of purification BITEROJIENIN prepared in the molecular-weight-measurement example 1 of BITEROJIENIN (1) was measured with the electrophoresis method. SDS-7.5% polyacrylamide-gel [presentation: 7.3% acrylamide, 0.2% methylenebis acrylamide, 0.1%SDS, a 0.35M tris-hydrochloric acid (pH 6.8), and] (the reagent which purchased all from Wako Pure Chem Industries is used) were produced. Next, after mixing purification BITEROJIENIN (5microl) of 0.5microg, and Tris-SDS-BME SepraSol [0.0625M The sample processing liquid:first chemicals incorporated company make which consists of a tris-hydrochloric acid (pH 6.8), 2%SDS, 10% glycerol, 5%beta-mercaptoethanol, and a 0.001% bromophenol blue (BPB)] of 5microl and keeping it warm for 5 minutes at 50 degrees C, one well of said gel was presented with the whole quantity. Moreover, the next well was similarly presented with amount marker of protein molecules (protein molecular-weight-marker "the first" II: first chemicals incorporated company make) 5microl which consists of a myosin (molecular-weight 200kDa), beta-galactosidase (116kDa), bovine serum albumin (66kDa), aldolase (42.4kDa), carbonic anhydrase (30kDa), and a myoglobin (17.2kDa). Electrophoresis of this gel was carried out in 40mA constant current for about 1 hour using the migration buffer which consists of a 0.025M tris-hydrochloric acid (pH 8.4), 0.1% SDS, and 0.192% glycerol. After migration, after CBB-R250 stain solution (the first chemicals incorporated company make) dyed gel for about 1 hour, it decolorized overnight using the decolorization liquid which consists of isopropyl alcohol and a 10% (V/V) acetic acid 10% (V/V). A result is shown in drawing 16 . By comparing the location of the detected band with the location of the band of molecular weight marker protein, the molecular weight of the appearance of purification BITEROJIENIN under reduction conditions was measured as they are about 200 kDa(s).
[0013] Example 2 (analysis of the amino-terminus amino acid sequence of BITEROJIENIN)
The amino-terminus amino acid sequence of purification BITEROJIENIN prepared in the example 1 (1) was analyzed using the amino acid sequencer. Consequently, the amino acid sequence shown by the array number 1 was determined. When compared with the amino acid sequence of BITEROJIENIN of Fundulus heteroclitus (one kind of teleosts) to which this array is already reported, 13 amino acid was in agreement among 16 amino acid.
[0014] Example 3 (production of an anti-BITEROJIENIN antibody)
BITEROJIENIN of the HIMEDAKA origin prepared in the example 1 (1) was mixed with the Freund perfect AJU band, and hypodermically [of a rabbit [KBL:JW rabbit 9 weeks old, a male, and weight 1.80 kg (at the time / 2.53 kg / of experiment termination)] / regions-of-back] was medicated. The dose was made into 1.10 mg protein / rabbit, and was prescribed for the patient a total of 4 times 3 times every two weeks after first time administration. Whole blood extraction was carried out 52 days after the priming, and about 53ml blood serum was obtained. This was used for future experiments as an anti-BITEROJIENIN blood serum (it is hereafter described as antiserum.).
[0015] Example 4 (biotin-izing of antiserum)
Antiserum prepared in the example 3 was biotin-ized using the ECL Protein Biotinylation Module kit (product made from Amersham). The approach followed the attachment description of a kit. Each step is

as follows. When the quantum of the protein concentration of antiserum was carried out, they were 41.5 mg protein / ml. They are 40mM(s) about this. bicarbonate buffer (it attaches to said kit) It dilutes so that it may be set to 1mg protein / ml, and it is biotinylation of 120microl to this diluent 3 ml. The reagent was added (biotinylation reagent of 40microper 1mg protein l), and it shook at the room temperature for 1 hour. Next, after equilibrating Sephadex G25 column (1.5X8 cm) attached to the kit by PBS(-) (NISSUI PHARMACEUTICAL CO., LTD. make) 5 ml which contains BSA (product made from Sigma) 1.0%, the 2.5ml of the aforementioned biotin-ized reaction mixture is hung on this column, and subsequently it is 8 ml. PBS (-) was passed in this column. It collected 1ml of eluates at a time, and the quantum of the protein concentration of each fraction was carried out. Consequently, the protein concentration of each fraction was 0.013, 0.081, 0.28, 0.90, 1.63, 1.53 and 0.56, and 0.068mg/ml, respectively. The 4th - the 6th fractions were collected in one tube among these fractions (1.6 mg protein / ml). This was diluted with PBE (-), was saved at 4 degrees C so that it might be set to 1.0 mg protein / ml, and it was used for the following measurement as biotin-ized antiserum.

[0016] Example 5 (minute amount measuring method of BITEROJIENIN)

The protocol of a minute amount measuring method was created according to the antibody-sandwiches ELISA method (1997, the molecule biology experiment protocol II, and Maruzen Co., Ltd.) used for detection of a soluble antigen. Each step is as follows. each sample hole of the microplate (ICNBiomedicals, Inc., U.S.) of 96 wells -- 40 mM The antiserum diluted with bicarbonate buffer (ECL Protein Biotinylation Module kit; it attaches to the product made from Amersham) was added 50microl every, and it incubated at a night or 37 degrees C with the room temperature for 2 hours. subsequently, Tween 20 (product made from Bio-Rad) of 0.1(v/v) % is included using IMMUNOWASH (the product made from Bio-Rad, and MODEL 1575) After adding PBS (-) 200microl every to each sample hole, this was removed, and this washing actuation was repeated twice [further]. next, 1% After adding every [containing BSA (product made from Sigma) / PBS(-) 150microl] to each sample hole and incubating for 30 minutes at 37 degrees C, Tween 20 of 0.1(v/v) % is included. Each sample hole was washed 3 times by PBS(-) 200microl. then, after having diluted with PBS (-) purification BITEROJIENIN (antigen solution) prepared in the example 1 (1), adding to 100microl [every] sample hole and incubating at 37 degrees C for 1 hour, each sample hole was washed 3 times. Subsequently, the biotin-ized antiserum prepared in the example 4 was added every [100micro / l], and it incubated at 37 degrees C for 1 hour. Like each above-mentioned step, each sample hole was washed, streptoavidin-HRP diluted with PBS (-) was added every [100micro / l], and it incubated at 37 degrees C for 1 hour. Furthermore, luminous reaction liquid [4 mM O-phenylene diamine after washing each sample hole 3 times like each step (Wako Pure Chem), 0.004% H2O2 (Wako Pure Chem), 0.02M citric acid (Nakarai Tesuku), 0.05 -- M Na2HPO4.12H2O (Kanto chemistry)] 100microl [every] each sample hole -- adding -- After incubating for 10 minutes at 37 degrees C, the reaction stop solution [0.1M H2SO4 (Kanto chemistry)] was added 50microl every. To the last, he is a microplate reader. (the product made from Bio-Rad, MICROPLATE READER, Benchmark) It used and the absorbance of 490nm in each sample hole was measured.

[0017] Example 6 (condition examination of a minute amount measuring method)

In the measuring method shown in the example 5, in order to examine conditions, such as an addition of the solid-phase-ized antiserum, a dilution scale factor of biotin-ized antiserum, and a dilution scale factor of streptoavidin-HRP, the following experiments were conducted. First, in order to determine the amount of antisera which was suitable for solid-phase-izing on a plate in the phase of the beginning of this measuring method, the amount of the antigen (purification BITEROJIENIN) added to the system of reaction, the amount of biotin-ized antiserum, and the amount of streptoavidin-HRP were fixed, and it experimented by changing the addition of antiserum. The amount of each component added to each well is as follows. Biotin-ized antiserum 100microl diluted with PBS (-) 500 times, purification BITEROJIENIN 4 ng protein, streptoavidin diluted with PBS (-) 1500 times - The antiserum of HRP100microl and 10 - 500 ng protein was added to each sample hole. A result is shown in drawing 2 . This experiment was conducted 3 times and repeatability was accepted. Consequently, even if it solid-phase-ized antiserum more than 250 ng protein, the absorbance of 490 nm seldom increased. The amount of antisera applied in order to solid-phase-ize on a plate in this measuring method from this result was judged that 250 ng protein is desirable per one well. Next, in order to examine the addition of the biotin-ized antiserum in this measuring method, the amount of the solid-phase-ized antiserum, the amount of an antigen (purification BITEROJIENIN), and the amount of streptoavidin-HRP were set constant, and it

experimented on the plate by changing the addition of biotin-ized antiserum. The amount of each component added to each well is as follows. 100micro of streptoavidin-HRP biotin-ized antisera I which were diluted with antiserum 250 ng protein, purification BITEROJIENIN 4 ng protein, and PBS (-) 1500 times and which were diluted with I and PBS (-) 3000 times to 50 times 100 micro was added to each sample hole. The result was shown in drawing 3 . This experiment was conducted 3 times and repeatability was accepted. These experimental results showed that the absorbance of 490 nm increased in this system of measurement depending on the amount of the biotin-ized antiserum to add. However, when the dilution ratio of biotin-ized antiserum became 250 or less times, the inclination for a background value to become high was accepted, and it was judged that between 250 to 500 times was desirable as for the dilution ratio of the biotin-ized antiserum added in this system of measurement. Next, the protein of the body origin of a male cyprinodont investigated the effect which it has on system of measurement. A cyprinodont's length is as small as about 2cm order, and in order to require skill for extracting ascites and blood, after exposing a male cyprinodont to a chemical in the trial for evaluation of the estrogen Mr. activity of a chemical etc., it will be practical to use for a trial the sample which grinds the whole body and is obtained from now on. Then, it investigated whether the protein originating in the whole body of a male cyprinodont would affect this system of measurement. the male which is not exposed to the chemical which has estrogen Mr. activity -- an adult -- the sample containing the protein originating in the whole body was prepared using HIMEDAKA 10 individual. That is, after adding about 2ml [per one individual] PBS (-) and grinding with a homogenizer, centrifugal separation (the Hitachi Koki make, CR21 mold centrifuge) was carried out at 4 degrees C for 10 minutes by 10,000 rpm, supernatant liquid was taken and the quantum of the protein contained in this supernatant liquid was carried out. BITEROJIENIN concentration set constant the amount of the solid-phase-ized anti-BITEROJIENIN blood serum, the amount of biotin-ized antiserum, and the amount of streptoavidin-HRP, fluctuated the amount of the whole body origin protein of the male cyprinodont added in a system, and was measured. The amount of each component added to each well is as follows. The above-mentioned centrifugal supernatant liquid containing streptoavidin-HRP 100microl diluted with I and PBS (-) 1500 times 100micro of biotin-ized antisera diluted with anti-BITEROJIENIN blood serum 250 ng protein and PBS (-) 500 times, and protein 50 ng of the whole body origin of a male cyprinodont - 1microg was added to each sample hole. The result was shown in drawing 4 . This experiment was conducted twice and repeatability was accepted. The protein originating in the whole body of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity from the result of this experiment was judged not to affect not much greatly the system of measurement which used this invention antibody within measured limits.

[0018] Example 7 (production of a calibration curve)

The calibration curve for measuring BITEROJIENIN concentration was created using the measuring method examined in the example 6. The amount of each component added to each well is as follows. 10ng protein was added from streptoavidin-HRP 100microl diluted with antiserum 250 ng protein and PBS (-) 1500 times by I and PBS (-) 250 times or 100micro of biotin-ized antisera diluted 500 times, and purification BITEROJIENIN 100pg. The result was shown in drawing 5 .

[0019] Example 8 (purification of anti-BITEROJIENIN IgG)

Immunoglobulin G (it is hereafter described as IgG.) was refined among the antisera obtained in the example 3 using about 25ml. The step of purification is as follows. First, the ammonium-sulfate salting-out was performed about said antiserum, and the rough IgG fraction was obtained. Next, after having applied the aforementioned rough IgG fraction to this Protein A column after equilibrating a Protein A column by the equilibration buffer (3M NaCl, 1.5M glycine, pH 8.9), and passing a 10ml equilibration buffer, the elution buffer (0.1M citric acid, pH 4.0) was passed. After collecting the fractions by which elution was carried out and neutralizing by the elution buffer, this was condensed by the ammonium-sulfate salting-out, and it dialyzed by PBS further. The volume of the obtained IgG fraction was about 22ml, and protein concentration was 7mg/ml. It used for the trial after making this into anti-BITEROJIENIN IgG.

[0020] Example 9 (biotin-izing of anti-BITEROJIENIN IgG)

Anti-BITEROJIENIN IgG prepared in the example 8 was biotin-ized using the ECL Protein Biotinylation Module kit (product made from Amersham). The approach followed the attachment description of a kit. Each step is as follows. When the quantum of the protein concentration of anti-BITEROJIENIN IgG was

carried out, they were 7mg protein / ml. They are 40mM(s) about this. bicarbonate buffer (it attaches to said kit) It dilutes so that it may be set to 1mg protein / ml, and it is biotinylation of 120microl to this diluent 3 ml. The reagent was added (biotinylation reagent of 40microper 1mg protein l), and it shook at the room temperature for 1 hour. Next, after equilibrating Sephadex G25 column (1.5X8 cm) attached to the kit by PBS(-) (NISSUI PHARMACEUTICAL) 5 ml which contains BSA (Sigma) 1.0%, the 2.5ml of the aforementioned biotin-ized reaction mixture is hung on a column, and subsequently it is 8 ml. PBS (-) was passed in this column. It collected 1ml of eluates at a time, and the quantum of the protein concentration of each fraction was carried out. consequently, the protein concentration of each fraction -- respectively -- 0, 0, 0.23, and 0. -- 75, 1.1, and 0. -- they were 9, 0.23, and 0mg/ml. The 4th - the 6th fractions were collected in one tube among these fractions. The protein concentration of the collected fractions was 0.75 mg protein / ml. This was saved at 4 degrees C and it used for future measurement as biotin-ized anti-BITEROJIENIN IgG (it is hereafter described as biotin-ized IgG.).

[0021] Example 10 (minute amount measuring method of BITEROJIENIN)

The next experiment was conducted using anti-BITEROJIENIN IgG prepared in the example 8. The protocol of a minute amount measuring method was created according to the antibody-sandwiches ELISA method (1997, the molecule biology experiment protocol II, and Maruzen Co., Ltd.) used for detection of a soluble antigen. Each step is as follows. each sample hole of the microplate (ICNBiomedicals, Inc., U.S.) of 96 wells -- 40 mM anti-BITEROJIENIN IgG diluted with bicarbonate buffer (ECL Protein Biotinylation Module kit; it attaches to the product made from Amersham) -- every [50microl] -- adding -- a room temperature -- a night or 37 degrees C It incubated for 2 hours. subsequently, Tween 20 (product made from Bio-Rad) of 0.1(v/v) % is included using IMMUNOWASH (the product made from Bio-Rad, and MODEL 1575) After adding PBS (-) 200microl every to each sample hole, this was removed, and this washing actuation was repeated twice [further]. next, 1% After adding every [containing BSA (product made from Sigma) / PBS(-) 150microl] to each sample hole and incubating for 30 minutes at 37 degrees C, Tween 20 of 0.1(v/v) % is included. Each sample hole was washed 3 times by PBS(-) 200microl. then, after having diluted with PBS (-) purification BITEROJIENIN (antigen solution) prepared in the example 1 (1), adding to 100microl [every] sample hole and incubating at 37 degrees C for 1 hour, each sample hole was washed 3 times. Subsequently, biotin-ized IgG prepared in the example 9 was diluted with PBS (-), and it added every [100micro / l], and incubated at 37 degrees C for 1 hour. Like each above-mentioned step, each sample hole was washed, streptoavidin-HRP diluted with PBS (-) was added every [100micro / l], and it incubated at 37 degrees C for 1 hour. Furthermore, luminous reaction liquid [4 mM O-phenylene diamine after washing each sample hole 3 times like each step (Wako Pure Chem), 0.004% H2O2 (Wako Pure Chem), 0.02M citric acid (Nakarai Tesuku), 0.05 -- M Na2HPO4.12H2O (Kanto chemistry)] 100microl [every] each sample hole -- adding -- After incubating for 10 minutes at 37 degrees C, the reaction stop solution [0.1M H2SO4 (Kanto chemistry)] was added 50microl every. To the last, he is a microplate reader. (the product made from Bio-Rad, MICROPLATE READER, Benchmark) It used and the absorbance of 490nm in each sample hole was measured.

[0022] Example 11 (condition examination of a minute amount measuring method)

In the measuring method shown in the example 10, in order to examine conditions, such as a solid-phase-ized addition of anti-BITEROJIENIN IgG, a dilution scale factor of biotin-ized IgG, and a dilution scale factor of streptoavidin-HRP, the following experiments were conducted. First, in order to determine the amount of anti-BITEROJIENIN IgG which was suitable for solid-phase-izing on a plate in the phase of the beginning of this measuring method, the amount of the antigen (purification BITEROJIENIN) added to the system of reaction, the amount of biotin-ized IgG, and the amount of streptoavidin-HRP were fixed, and it experimented by changing the addition of anti-BITEROJIENIN IgG. The amount of each component added to each well is as follows. Streptoavidin-HRP 100microl diluted with biotin-ized IgG 100micro[of 1ng protein / mul] l, purification BITEROJIENIN 5 ng protein, and PBS (-) 1500 times and anti-BITEROJIENIN IgG 1-300ng protein were added to each sample hole. A result is shown in drawing 6 . This experiment was conducted twice and repeatability was accepted. Consequently, even if it solid-phase-ized anti-BITEROJIENIN IgG more than 150 ng protein, the absorbance of 490nm seldom increased. As for the amount of anti-BITEROJIENIN IgG applied in order to solid-phase-ize on a plate in this measuring method from this result, 150ng protein was judged to be desirable from per [100] one well. Next, in order to examine the addition of biotin-ized IgG in this

measuring method, the solid-phase-ized amount of anti-BITEROJIENIN IgG, the amount of an antigen (purification BITEROJIENIN), and the amount of streptoavidin-HRP were set constant, and it experimented on the plate by changing the addition of biotin-ized IgG. The amount of each component added to each well is as follows. Anti-BITEROJIENIN IgG 150 ng protein, purification BITEROJIENIN Streptoavidin-HRP 100microl diluted with 5 ng protein and PBS (-) 1500 times and biotin-ized IgG 1-300 ng protein were added to each sample hole. The result was shown in drawing 7 . This experiment was conducted twice and repeatability was accepted. These experimental results showed that the absorbance of 490 nm increased in this system of measurement depending on the amount of biotin-ized IgG to add. However, even if it added biotin-ized IgG more than 75ng protein, the absorbance of 490 nm seldom increased. It was judged that between 50 to 75ng protein was more desirable than this result as for the addition of biotin-ized IgG added in this system of measurement. Next, the protein of the body origin of a male cyprinodont investigated the effect which it has on system of measurement. the male which is not exposed to the chemical which has estrogen Mr. activity -- an adult -- the sample containing the protein originating in the whole body was prepared using HIMEDAKA 10 individual. That is, after adding about 2ml [per one individual] PBS (-) and grinding with a homogenizer, centrifugal separation (the Hitachi Koki Co., Ltd. make, CR21 mold centrifuge) was carried out at 4 degrees C for 10 minutes by 10,000 rpm, supernatant liquid was taken and the quantum of the protein contained in this supernatant liquid was carried out. Measurement of BITEROJIENIN concentration set constant the solid-phase-ized amount of anti-BITEROJIENIN IgG, the amount of biotin-ized IgG, and the amount of streptoavidin-HRP, and was performed by fluctuating the amount of the whole body origin protein of the male cyprinodont added in a system. The amount of each component added to each well is as follows. The above-mentioned centrifugal supernatant liquid 50ng(s)-200microg Containing the protein of the whole body origin of a male cyprinodont which is not exposed to the chemical which has streptoavidin-HRP 100microl and estrogen Mr. activity which were diluted with anti-BITEROJIENIN IgG 150ng protein and PBS (biotin-ized IgG 100microl diluted with -) 75 times, PBS(-) 1500 times was added to each sample hole. The result was shown in drawing 8 -10.

[0023] Example 12 (production of a calibration curve)

The calibration curve for measuring the amount of BITEROJIENIN was created using the measuring method examined in the example 11. The amount of each component added to each well is as follows. Are streptoavidin-HRP 100microl diluted with anti-BITEROJIENIN IgG 150ng protein, biotin-ized IgG 75ng protein, and PBS (-) 1500 times, and it is purification BITEROJIENIN. 20ng protein was added from 100pg. The result was shown in drawing 11 . Furthermore, the calibration curve of BITEROJIENIN was created using purification BITEROJIENIN on the desirable conditions determined as the bottom of existence of protein 2microg originating in the whole body of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity, or 10microg in the example 11. BITEROJIENIN concentration was measured in the range of 20ng protein from 100pg(s). The result was shown in drawing 12 and drawing 13 .

[0024] Example 13 (measurement of the estrogen Mr. activity of a chemical)

The amount of generation of BITEROJIENIN in the cyprinodont of the male exposed to beta-estradiol or nonyl phenol was measured. beta-estradiol (Wako Pure Chem make) which dissolved in dimethylformamide was added to dechlorination water, and the water solution of beta-estradiol was prepared. beta-estradiol concentration in this water solution made maximum density 1microg/l, and set up the concentration of 1microg/l, 0.1microg/l, 0.01microg/l, and 0.001microg/l. In addition, each dimethylformamide concentration in this water solution was prepared so that it might become 100microl/l. the inside of these beta-estradiol water solutions -- a male -- an adult -- every 35 HIMEDAKA per 1 concentration was bred, and it extracted five cyprinodonts at a time 1, 3, 5, 7, 10, 14, and 21 days after from initiation of this breeding. Extracted cyprinodont 1 individual hit PBS (-) (NISSUI PHARMACEUTICAL CO., LTD. make) About 2-2.5ml was added and it ground with the homogenizer. At-long-intervals alignment separation (Hitachi Koki [Co., Ltd.] make: CR21 mold centrifuge) of the obtained grinding liquid was carried out by 4 degrees C and 10,000rpm for 10 minutes, supernatant liquid was collected, centrifugal separation of this was further carried out to the above on these conditions again, and supernatant liquid was collected. moreover, the dechlorination water which contains 100microl. [/l.] dimethylformamide like the above -- a male -- an adult -- HIMEDAKA was bred, the grinding liquid was prepared and the centrifugal supernatant liquid of this grinding liquid was obtained.

Thus, grinding liquid centrifugal supernatant liquid of male HIMEDAKA bred in prepared beta-estradiol water solution To 5, 10, 25, 50, or a 100ng protein considerable amount, PBS (-) addition of the grinding liquid centrifugal supernatant liquid of male HIMEDAKA bred in the dimethylformamide water solution was carried out so that total protein mass might serve as 100ng(s), and the total capacity might be set to 100microl in addition, and the sample solution was prepared. Thus, the ELISA method was performed using anti-BITEROJIENIN IgG on the desirable conditions examined in the example 11 about prepared sample solution 100microl according to the approach indicated in the example 10, the absorbance of 490nm was measured, and the quantum of BITEROJIENIN in a sample solution was carried out. In this ELISA method, about the same sample, it used four wells at a time, and measured, and the average was computed. A result is shown in drawing 14 . In addition, the calibration curve for calculating the amount of BITEROJIENIN was created like the example 12 from the absorbance of 490nm under the 100ng protein considerable-amount existence of the grinding liquid centrifugal supernatant liquid of male HIMEDAKA bred in the above-mentioned dimethylformamide water solution. the same approach -- the water solution of nonyl phenol -- a male -- an adult -- HIMEDAKA was bred and the amount of BITEROJIENIN of this cyprinodont inside of the body was measured. Concentration in the water solution of the nonyl phenol used for breeding was made into 100microg/l, 10microg/l, and 1microg/l. A result is shown in drawing 15 .

[0025]

[Effect of the Invention] Offer of an approach, this kit for BITEROJIENIN detection, etc. which carry out the quantum of BITEROJIENIN in the antibody to BITEROJIENIN of the Cyprinodontiformes animal origin useful in order to evaluate the estrogen Mr. activity of a chemical, and this BITEROJIENIN, and a sample by this invention is attained.

[0026]

[Layout Table]

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[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the elution pattern from the TSK-GEL G3000SW column in the purification process of BITEROJIENIN of this invention.
[Drawing 2] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result of having changed and examined the solid-phase-ized amount of antisera (antiserum use as an anti-BITEROJIENIN antibody).
[Drawing 3] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result of having changed and examined the dilution ratio of the biotin-ized antiserum added to system of measurement (biotin-ized antiserum is used as a biotin-ized antibody).
[Drawing 4] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result which the protein of the body origin of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity was made to live together, and was examined (antiserum use as an anti-BITEROJIENIN antibody).
[Drawing 5] It is drawing showing the calibration curve of BITEROJIENIN created by the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention (antiserum use as an anti-BITEROJIENIN antibody).
[Drawing 6] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result of having changed and examined the solid-phase-ized amount of anti-BITEROJIENIN IgG (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).
[Drawing 7] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result of having changed and examined the amount of biotin-ized IgG added to system of measurement (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).
[Drawing 8] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result which the protein of the body origin of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity was made to live together, and was examined (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).
[Drawing 9] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result which the protein of the body origin of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity was made to live together, and was examined (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).
[Drawing 10] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the result which the protein of the body origin of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity was made to live together, and was examined (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).
[Drawing 11] It is drawing showing the calibration curve of BITEROJIENIN created by the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).
[Drawing 12] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the calibration curve of created BITEROJIENIN under the condition which made 2micro [of protein] g / well of the body origin of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity live together (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).

[Drawing 13] In the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention, it is drawing showing the calibration curve of created BITEROJIENIN under the condition which made 10micro [of protein] g / well of the body origin of a male cyprinodont which is not exposed to the chemical which has estrogen Mr. activity live together (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody).

[Drawing 14] It is drawing showing the result of having measured the amount of generation of BITEROJIENIN in the male cyprinodont exposed and bred to beta-estradiol by the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody). <> The amount of generation of BITEROJIENIN in the male cyprinodont bred in beta-estradiol water solution of 0.001microg/l is shown. ** The amount of generation of BITEROJIENIN in the male cyprinodont bred in beta-estradiol water solution of 0.01microg/l is shown. A black trigonum shows the amount of generation of BITEROJIENIN in the male cyprinodont bred in 0.1microg [/l.] beta-estradiol water solution, and - shows the amount of generation of BITEROJIENIN in the male cyprinodont bred in beta-estradiol water solution of 1microg/l.

[Drawing 15] It is drawing showing the result of having measured the amount of generation of BITEROJIENIN in the male cyprinodont exposed and bred to nonyl phenol by the quantum approach of BITEROJIENIN using the anti-BITEROJIENIN antibody of this invention (anti-BITEROJIENIN IgG use as an anti-BITEROJIENIN antibody). <> The amount of generation of BITEROJIENIN in the male cyprinodont bred in the nonyl phenol water solution of 1microg/l is shown, ** shows the amount of generation of BITEROJIENIN in the male cyprinodont bred in the nonyl phenol water solution of 10microg/l, and a black trigonum shows the amount of generation of BITEROJIENIN in the male cyprinodont bred in the nonyl phenol water solution of 100microg/l.

[Drawing 16] It is drawing showing the result of having carried out electrophoresis of purification BITEROJIENIN and the molecular weight marker protein in SDS-7.5% polyacrylamide gel. The left-hand side lane was presented with purification BITEROJIENIN, and the right-hand side lane was presented with molecular weight marker protein. A right-hand side figure shows the molecular weight of each marker protein.

[Translation done.]

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2.**** shows the word which can not be translated.
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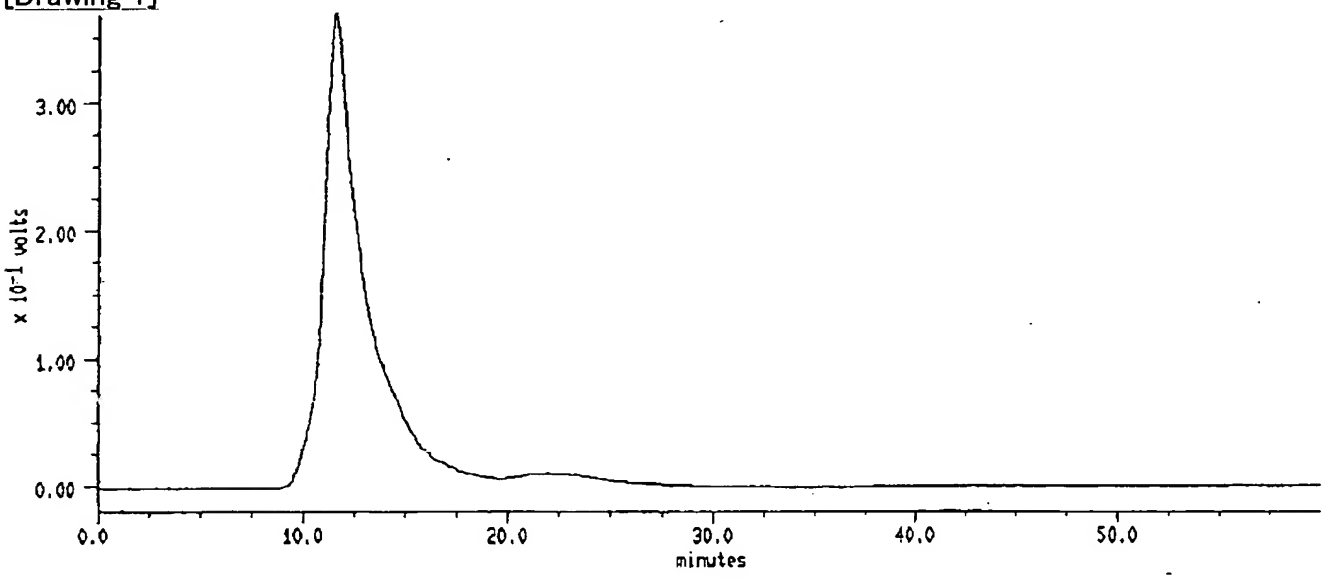
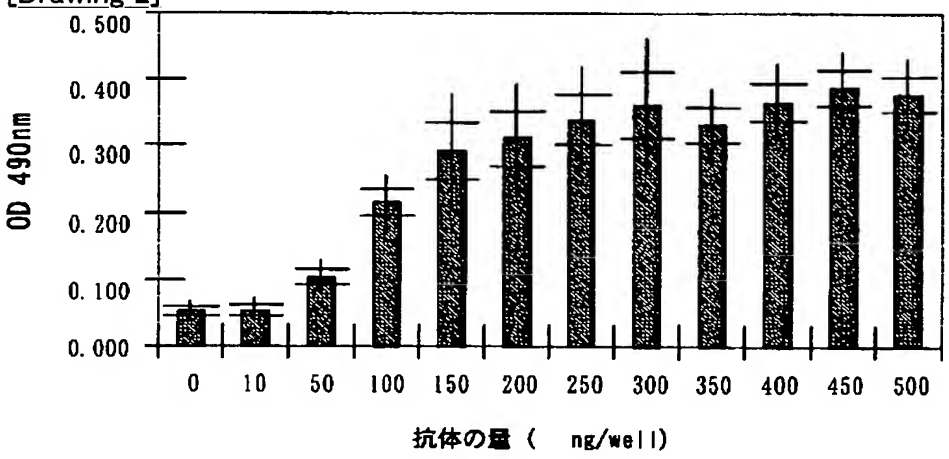
DRAWINGS

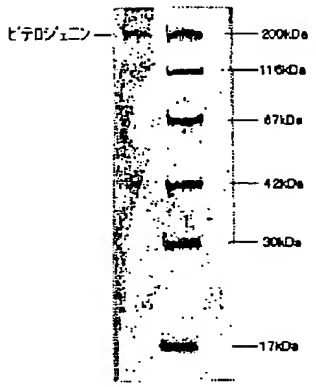
[Drawing 1]



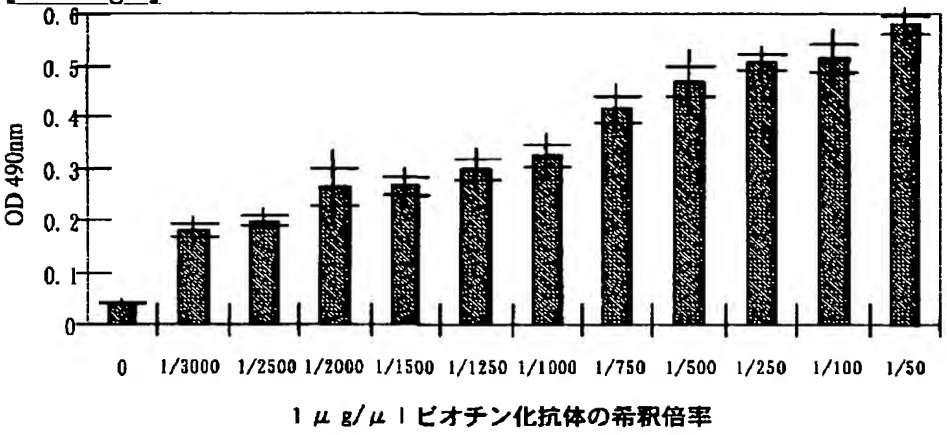
[Drawing 2]



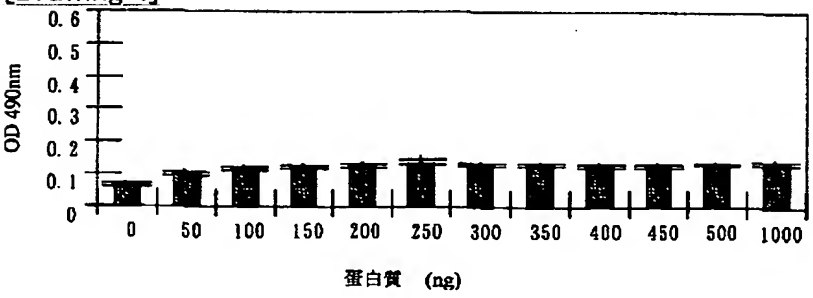
[Drawing 16]



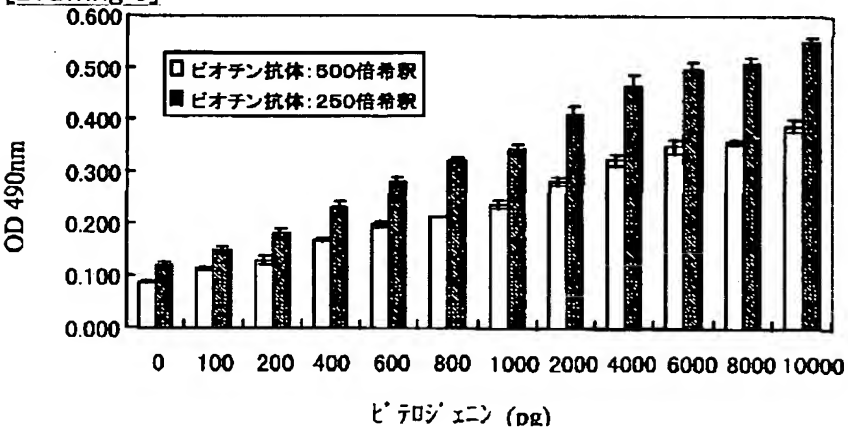
[Drawing 3]



[Drawing 4]



[Drawing 5]



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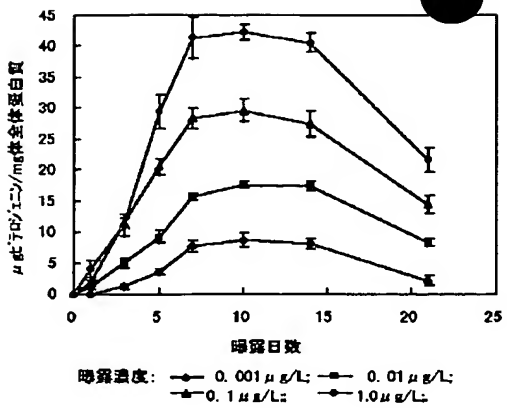

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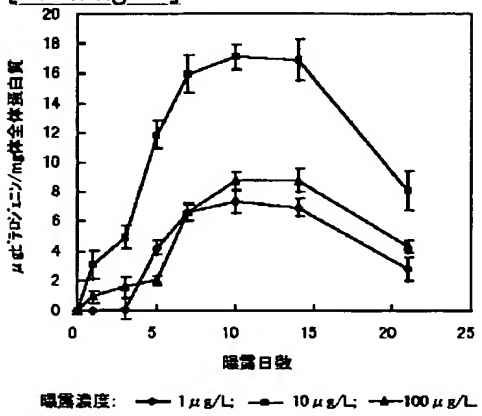

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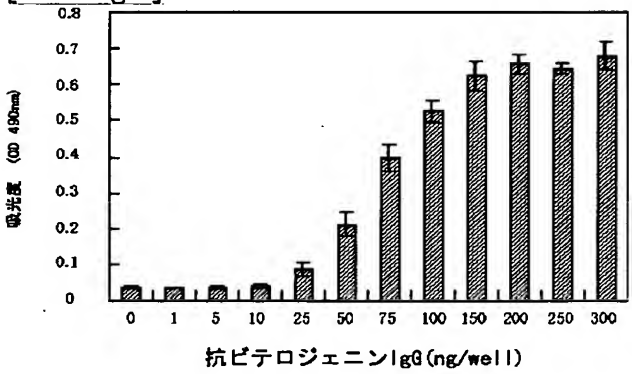

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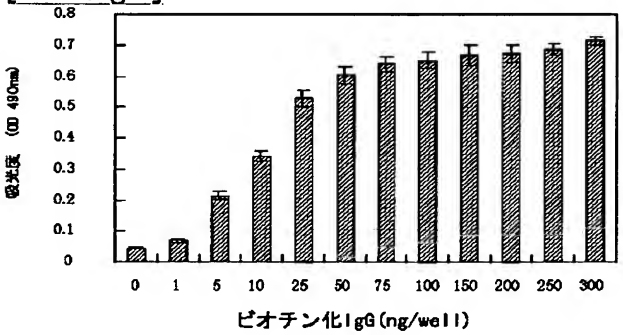

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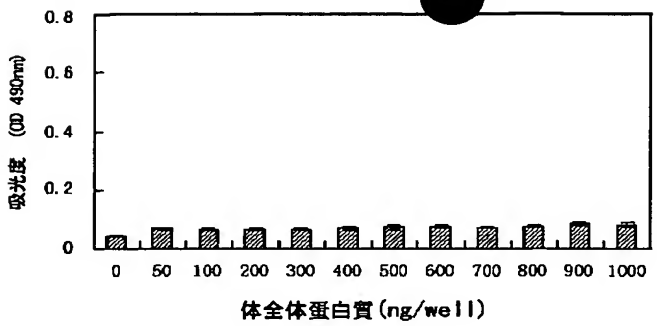
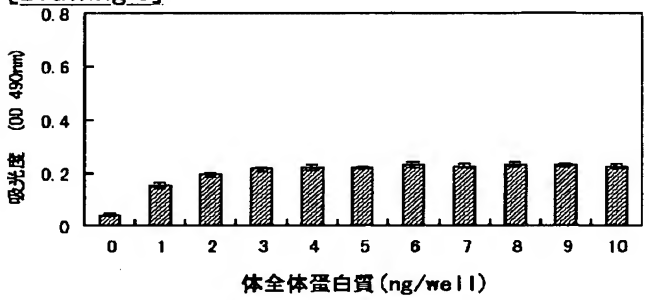
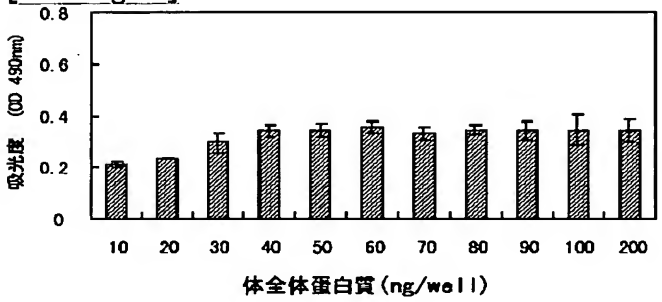


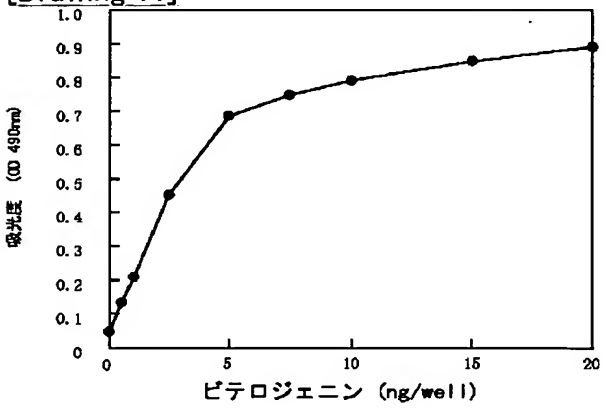
[Drawing 9]



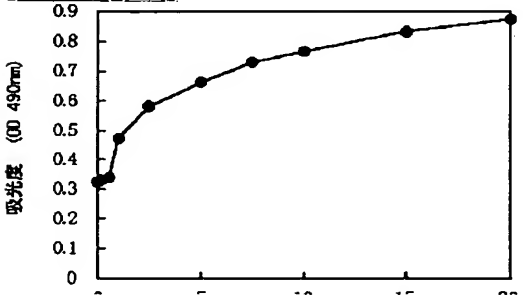
[Drawing 10]

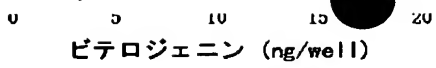


[Drawing 11]

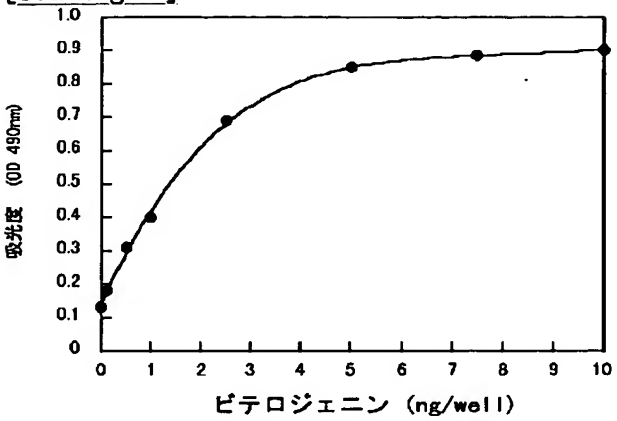


[Drawing 13]





[Drawing 12]



[Translation done.]